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ARTICLE

Exogenous Gibberellic Acid Reprograms Soybean to Higher Growth and Salt Stress Tolerance

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The agricultural industry is severely affected by salinity due to its high magnitude of adverse impacts and worldwide distribution. We observed the role of exogenous gibberellic acid (GA_3) in salinity alleviation of soybean. We found that GA_3 application significantly promoted plant length and plant fresh/dry biomass while markedly hindered by NaCl induced salt stress. The adverse effect of salt stress was mitigated by GA_3, as growth attributes significantly recovered, when GA_3 was added to salt stressed soybean plants. Elevated GA_3 treatments increased daidzein and genistein contents (commonly known as phytoestrogens) of soybean leaves under control and salt stress conditions. Phytohormonal analysis of soybean showed that the level of bioactive gibberellins (GA_1 and GA_4) and jasmonic acid increased in GA_3 treated plants, while the endogenous abscisic acid and salicylic acid contents declined under the same treatment. GA_3 mitigated the adverse effects of salt stress by regulating the level of phytohormones, thus aiding the plant in resuming its normal growth and development. The presence of GA_1 and GA_4 showed that both early-C13-hydroxylation and non-C13-hydroxylation pathways of GA biosynthesis are functional in soybean. It was concluded that GA_3 ameliorates the adverse effects of salt stress and restores normal growth and development of soybean.

KEYWORDS: Soybean; gibberellic acid; salinity stress; jasmonic acid; salicylic acid; abscisic acid

INTRODUCTION

The human population continues to grow, whereas the size of arable land is on decline as it is rendered unfavorable for cultivation due to various environmental constraints, and greater emphasis must be given to bringing marginally productive and nonarable land under production. Salinity of soil and water is a major problem that restricts yield on almost 40 million hectares of irrigated land, which is approximately one-third of the irrigated land on earth (*1*). It was estimated that about 50% of the arable land will be affected by salt stress by the year 2050 (*2*). Saline soils possess high levels of sodium (Na^+) and chloride (Cl^-) contents and thus exert adverse abiotic stress on plants. Scientists are working on the reclamation of saline lands, and much attention is now focused on the use of plant growth regulators, which are known to be involved in the regulation of plant responses to the external environment and to control a number of stress-induced genes (*3*).

Salt stress disturbs normal growth and development of plants by altering physiological and biochemical processes. The response of plants to salt stress depends on multiple factors, but phytohormones are thought to be the most important endogenous substances involved in the mechanisms of tolerance or susceptibility of plants (*4*). Many of the proteins produced by the plant under abiotic stress are induced by phytohormones, such as abscisic acid (*5*), salicylic acid (*6*), and jasmonates (*7*). These plant growth hormones are usually present in minute quantities but play a pivotal role in growth and development processes. For instance, gibberellins (GAs) affect stem elongation, flowering, fruit development, and seed germination (*8*), while abscisic acid (ABA) being a generic stress hormone is upregulated by salinity and induces genes involved in salt and osmotic alleviation (*9*). Jasmonic acid (JA) influences seed germination, root growth, fertility, fruit ripening, and senescence (*10*, *11*), and activates plant defense mechanisms in response to insect-driven wounding, pathogens, and environmental stresses including drought, low temperature, and salinity (*12*). Salicylic acid (SA) is an important signal substance that induces systemic acquired resistance (SAR) against pathogen attack on plants (*13*).

Soybean is an important source of nutrition in the world and is popularly known as a healthy food in many Asian countries. Soybean crop is grown for oil production, and a smaller percentage of soybeans are used directly for human consumption. In China, Japan, and Korea, soybean and products made from the bean (miso, natto, tofu, douchi, doenjang, chungkookjang, ganjang, and others) are part of the daily diet. In Korean cuisine, soybean sprouts (kongnamul) are used in a variety of dishes.

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Currently, there is an increasing consumption of soybean worldwide due to its nutritional properties and the beneficial characteristics of its constituent compounds such as isoflavone. Consumption of isoflavone is associated with human health benefits such as decreased risk of heart disease, menopausal symptoms, cardiovascular disease, and bone resorption as well as breast, prostate, and colon cancers (*14*, *15*). The physiological function of isoflavone is mediated by a variety of mechanisms including estrogenic activity as well as inhibition of topoisomerase and protein kinases (*16*). Daidzein and genistein are important chemical constituents during the isoflavone biosynthetic pathway and are regarded as phytoestrogens due to their pivotal role in human health (*17*).

Soybean is generally considered to be salt-sensitive (*18*), and soybean plants grown in saline conditions exhibit symptoms of leaf chlorosis, stunting, and biomass reduction due to chloride induced toxicity (*19*). Therefore, there is a need to counteract the adverse effects of salt stress and boost soybean performance even under saline conditions. Attention has now been focused on the use of plant growth regulators, such as gibberellic acid, which are known to be important in the regulation of plant responses to the external environment and to control a number of stress-induced genes (*3*). Previous research has confirmed the potential of gibberellic acid (GA_3) in improving crop performance under normal conditions. However, very little has been known on the favorable role of GA_3 in saline conditions. In the current study, we investigated sole and interactive effects of GA_3 and NaCl on the growth characteristics of hydroponically grown soybean cv. Hwangkeum. Furthermore, pertinent changes in endogenous daidzein and genistein contents and levels of endogenous phytohormones in response to GA_3 and NaCl application were also studied.

MATERIALS AND METHODS

Plant Material and Growth Conditions. Seeds of soybean cultivar Hwangkeum were procured from Rural Development Administration (RDA), South Korea. Seeds were surface sterilized with 5% sodium hypochlorite (NaOCl) for 15 min, thoroughly rinsed with distilled H_2O, and sown in plastic pots filled with perlite, under greenhouse conditions. Modified Hoagland solution containing 5 mM KNO_3, 1 mM NH_4NO_3, 0.5 mM KH_2PO_4, 5 mM $Ca(NO_3)_2 \cdot 4H_2O$, 1.5 mM Fe-EDTA, and 2 mM $MgSO_4 \cdot 7H_2O$, and other micronutrients in their original concentration were used as the fertilizer (*20*). Gibberellic acid and NaCl treatments to soybean seedlings were started after 17 days of sowing (17 DAS). GA_3 was applied in three concentrations (0.5 μM, 1.0 μM, and 5.0 μM), while each plastic pot containing three seedlings received 50 mL of GA_3 solution. For salt stress induction, a single dose of 400 mL of NaCl solution (100 mM strength) was given to each plastic pot. Soybean plants were harvested after 30 days of sowing. The experiment was a randomized complete block design (RCBD), consisting of 8 treatments and each treatment with three replicates.

Measurement of Growth Characteristics. The plant growth attributes, i.e., plant length and shoot and root fresh and dry weights were measured at harvest time, while chlorophyll content of fully expanded leaves was analyzed with the help of a chlorophyll meter (Minolta Co., Ltd., Japan) just before harvest. We randomly selected 18 plants per treatment for measuring soybean growth components. The dry weight was measured after drying the samples at 70 °C for 48 h in an oven (*21*).

Isoflavone Analysis. Daizein and genistein contents of soybean leaves were extracted and analyzed by the standard methods in refs *22* and *36*. Dried powder (0.2 g) was added to 10 mL of 80% EtOH and incubated in an Ultrasonic bath (Kodo Co., Korea) at 50 °C for an hour. Samples were placed in a shaking incubator (150 rpm) at 50 °C for 15 h and then passed through a 0.45 μm syringe filter. The filtered sample (10 μL) was injected using gradient solutions viz. acetonitrile and 0.1% of acetic acid in water. The HPLC system consisted of TOTALCHROM V6.2.0.0.1 with an LC Instrument control (PerkinElmer series 200, USA) and a COL-CHOICE C18 packed column, 4.6 mm $\times$150 mm (5 μm). The solvent flow rate was 1.0 mL/min. The elution was monitored by UV-absorption (Series 200 UV/vis Detector) at 260 nm. Identification of the isoflavone was based on comparisons with retention times of genuine standards (daidzein and genistein) (Sigma Chemical Co, USA).

GA Analysis. Plants were harvested after 24 h treatments, immediately frozen in liquid nitrogen, and stored in a refrigerator (Sanyo-ultra low, Japan) at −70 °C. The samples comprising whole shoot were lyophilized and crushed to powder form. A 0.5 g lyophilized sample was used for GA analysis each time. The extraction and quantification of endogenous bioactive GA_1 and GA_4 were carried out by following an established protocol (*23*). The GC (Hewlett-Packard 6890, 5973N Mass Selective Detector) with a HA-1 capillary column (30 m $\times$ 0.25 mm i.d., 0.25 μm film thickness) was programmed with an oven temperature at 60 °C for 1 min, then with a rise of 15 °C min^{-1} to 200 °C, and followed by 5 °C min^{-1} to 285 °C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a mass selective detector with an interface and source temperature of 280 °C, an ionizing voltage of 70 eV, and a dwell time of 100 ms. Full scan mode (the first trial) and three major ions of the supplemented [$^2H_2$] GA internal standards (the second trial) and the endogenous gibberellins were monitored simultaneously (standard GAs were purchased from Professor Lewis N. Mander, Australian National University, Canberra, Australia). The endogenous GA contents of GA_1 and GA_4 were calculated from the peak area ratios of 506/508 and 284/286, respectively. The data was calculated in nanograms per gram dry weight, and the analysis was repeated three times, using different samples each time.

ABA Analysis. The endogenous free ABA contents of soybean shoots were quantified following previous protocols (*24*, *25*). The lyophilized and ground samples (0.5 g) were extracted with 30 mL of extraction solution containing 95% isopropanol, 5% glacial acetic acid, and 100 ng of [(±)-3,5,5,7,7,7-d6]- ABA. The filtrate was concentrated with a rotary evaporator. The residue was dissolved in 4 mL of 1 N NaOH solution and then washed three times with 3 mL of methylene chloride to remove lipophilic materials. The aqueous phase was brought to approximately pH 3.5 with 6 N HCl and partitioned three times into ethyl acetate (EtOAc). EtOAc extracts were then combined and evaporated. The dried residue was dissolved in phosphate buffer (pH 8.0) and then run through a polyvinylpolypyrrolidone (PVPP) column. The phosphate buffer was adjusted to pH 3.5 with 6 N HCl and partitioned three times into EtOAc. EtOAc extracts were combined again and evaporated. The residue was dissolved in dichloromethane, and passed through a silica cartridge (Sep-Pak; Water Associates, Milford, Massachusetts, USA) which was prewashed with 10 mL of diethyl ether/methanol (3:2, v/v) and 10 mL of dichloromethane. ABA was recovered from the cartridge by elution with 10 mL of diethyl ether/methanol (3:2, v/v). The extracts were dried and methylated by adding diazomethane for GC/MS-SIM (6890N network GC system and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA) analysis. For quantification, the Lab-Base (ThermoQuset, Manchester, UK) data system software was used to monitor responses to ions of *m*/*e* 162 and 190 for Me-ABA and 166 and 194 for Me-[$^2H_6$]-ABA. The data was calculated in nanograms per gram dry weight, and the analysis was repeated three times, using different samples each time.

JA Analysis. The endogenous JA contents of soybean shoots were quantified by following the modified protocol of McCloud and Baldwin (*26*). The lyophilized and samples were ground to a fine powder, and 0.1 g of it was suspended in a solution of acetone and 50 mM citric acid (70:30, v/v), while [9,10-$^2H_2$]-9,10-dihydro-JA (20 ng) was added as an internal standard. The extracts were allowed to evaporate overnight at room temperature to avoid losses of volatile fatty acids. The resulting aqueous solutions was then filtered and extracted with 3 $\times$ 10 mL of diethyl ether. The pooled extracts were then loaded on a solid phase extraction cartridge (500 mg of sorbent, aminopropyl). After loading, the cartridges were washed with 7.0 mL of trichloromethane and 2-propanol (2:1, v/v). The bound JA and the pertinent standard were eluted with 10 mL of diethyl ether and acetic acid (98:2, v/v). After the evaporation of solvents and esterification of the residue with excess diazomethane, the sample was adjusted to 50 μL with dichloromethane. The extracts were then analyzed by GC/MS (6890N network GC system and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA).

Table 1. Effect of Exogenous GA_3 and Salt Stress on Growth Characteristics of Soybean cv. Hwangkeumkong[a]

		plant length (cm)		shoot weight (g plant^{-1})		root weight (g plant^{-1})		chlophyll content
treatment (GA_3–μM)	NaCl (100 mM)	shoot	root	FW	DW	FW	DW	(plant^{-1})
control	−	46.26 ± 0.5 cd	37.43 ± 1 a	6.23 ± 0.2 cd	1.47 ± 0.06 c	9.48 ± 0.3 abc	0.87 ± 0.03 a	30.6 ± 0.4 a
	+	42.85 ± 0.6 d	36.56 ± 1.2 a	5.2 ± 0.4 d	1.04 ± 0.04 d	7.4 ± 0.2 c	0.57 ± 0.07 c	29.3 ± 0.08 a
0.5	−	48.0 ± 1.2 c	33.1 ± 2.8 a	6.7 ± 0.2 c	1.53 ± 0.08 bc	10.3 ± 0.5 ab	0.8 ± 0.04 bc	27.1 ± 0.7 a
	+	47.78 ± 0.5 c	37.81 ± 0.6 a	7.35 ± 0.6 bc	1.61 ± 0.1 bc	13.7 ± 1.6 a	1.07 ± 0.2 ab	28.8 ± 1.1 a
1.0	−	53.08 ± 2.6 ab	34.15 ± 3.0 a	7.41 ± 0.1 bc	1.81 ± 0.01 ab	9.76 ± 0.3 abc	0.91 ± 0.03 b	28.2 ± 1.4 a
	+	48.3 ± 0.6 c	41.08 ± 1.8 a	7.96 ± 0.2 ab	1.74 ± 0.08 abc	11.7 ± 0.3 ab	1.24 ± 0.06 a	28.5 ± 0.1 a
5.0	−	55.06 ± 0.9 a	35.3 ± 1.8 a	8.73 ± 0.3 a	1.91 ± 0.1 a	9.71 ± 0.9 abc	0.99 ± 0.05 ab	27.4 ± 1.5 a
	+	49.8 ± 0.2 bc	34.65 ± 1.2 a	8.01 ± 0.2 ab	1.68 ± 0.05 a	11.8 ± 0.1 ab	1.44 ± 0.02 a	28.4 ± 1.1 a

[a] In a column, treatment mean values with a common letter(s) are not significantly different at the 5% level by DMRT. FW stands for fresh weight, while DW stands for dry weight.

To enhance the sensitivity of the method, spectra were recorded in the selected ion mode, i.e., in the case of JA determination, monitoring the fragment ion at m/z = 83 amu corresponding to the base peaks of JA and [9,10-$^2H_2$]-9,10-dihydro-JA (*27*). The amounts of endogenous JA were calculated from the peak areas of JA in comparison with the corresponding standards. The data was calculated in nanograms per gram dry weight, and the analysis was repeated three times, using different samples each time.

SA Analysis. The endogenous free SA content was extracted and quantified, following the protocol in refs *28* and *29*. Lyphilized shoot samples were ground to powder, and 0.1 g was sequentially extracted with 90 and 100% methanol by centrifuging at 10000*g*. The combined methanol extracts were vacuum-dried. Dry pellets were resuspended in 2.5 mL of 5% trichloroacetic acid, and the supernatant was partitioned with ethyl acetate/cyclopentane/isopropanol (100:99:1, v/v). The top organic layer containing free SA was transferred to a 4 mL vial and dried with nitrogen gas. The dry SA was again suspended in 1 mL of 70% methanol. HPLC conditions were maintained in the fluorescence detector (Shimdzu RF-10AXL, with excitation 305 nm and emission 365 nm), and separation was done on a C18 reverse-phase HPLC column (Waters).

Statistical Analysis. The data was subjected to analysis of variance (ANOVA SAS, release 9.1; SAS, NC, USA) and Duncan's multiple range test (DMRT).

RESULTS

Influence of Gibberellic Acid on Soybean Growth Characteristics. In GA_3 applied treatments, shoot length and plant fresh and dry biomass of soybean were significantly promoted by elevated concentrations of GA_3. Maximum shoot length (55.06 cm), shoot fresh and dry biomass (8.73 and 1.91 g), and root dry weight (0.99 g) were recorded for 5.0 μM GA_3 treated plants (**Table 1**). NaCl application adversely affected the growth of soybean, as lowest values were recorded for 100 mM NaCl treated plants. However, an addition of GA_3 to such treatments significantly mitigated the adverse effects and provided promising results. In some parameters, i.e., root fresh and dry biomass (11.8 and 1.44 g), the results are even better than sole GA_3 applied treatments (**Table 1**). It was also observed that the chlorophyll and root length parameters were least effected by various treatments. An increase in exogenous GA_3 level caused an insignificant decline in the chlorophyll contents of soybean leaves.

Influence of Gibberellic Acid on Isoflavone Contents. Analysis of two important phytoestrogens, i.e., daidzein and genistein showed that GA_3 and salinity significantly affected their levels in soybean leaves (**Table 2**). Salt stress significantly decreased the levels of daidzein and genistein, while GA_3 application markedly improved the level of these valuable isoflavones. GA_3 application mitigated the adverse effects of salt stress on daidzein and genistein, as their contents increased with GA_3 under salt stress conditions. Under control conditions, maximum daidzein and genistein contents (279 μg/g) were found in plants treated with 1.0 μM GA_3, while 5.0 μM GA_3 treatment provided the best results (175.1 μg/g) under salt stress conditions. We also observed that daidzein was more abundant than genistein in soybean plants.

Table 2. Effect of Exogenous GA_3 and Salt Stress on Daizein and Genistein Contents of Soybean cv. Hwangkeumkong[a]

GA_3 treatment (μM)	NaCl (100 mM)	daidzein (μg/g)	genistein (μg/g)	total (μg/g)
control	−	78.3 ± 3.5	50.2 ± 9.1	128.5
	+	41.9 ± 1.7	27.3 ± 5.9	69.2
0.5	−	147.7 ± 5.4	76.6 ± 11.3	224.3
	+	64.2 ± 3.1	38.3 ± 5.9	102.5
1.0	−	185.2 ± 4.9	93.8 ± 10.2	279.0
	+	81.3 ± 5.7	61.4 ± 7.3	142.7
5.0	−	125.3 ± 7.4	98.5 ± 8.4	223.8
	+	98.7 ± 7.2	76.4 ± 7.1	175.1

[a] The Table shows mean values ± standard deviation (n = 3).

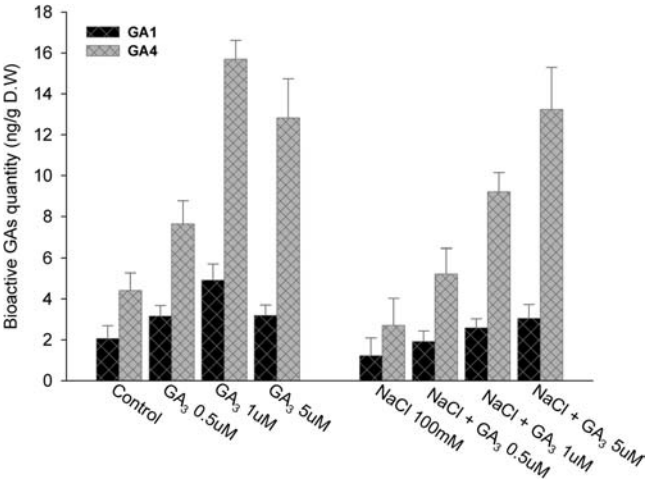

Figure 1. Level of endogenous bioactive GA_1 and GA_4 content of soybean shoots as influenced by exogenous GA_3 and NaCl.

Influence of Gibberellic Acid on Endogenous Bioactive GAs. Endogenous bioactive gibberellin (GA_1 and GA_4) contents of soybean were significantly promoted by elevated GA_3 application as maximum GA_1 and GA_4 contents (4.9 and 15.7 ng/g DW) were recorded in plants treated with 1.0 μM GA_3 (**Figure 1**). However, bioactive GA contents decreased in plants that received 5.0 μM GA_3. NaCl treatment significantly decreased endogenous bioactive GAs as lowest GA_1 and GA_4 contents (1.22 and 2.71 ng/g DW) were recorded in 100 mM NaCl treatments (**Figure 1**). An addition of GA_3 to salt stress plants considerably alleviated the effect of salt, and as a result, the level of endogenous bioactive

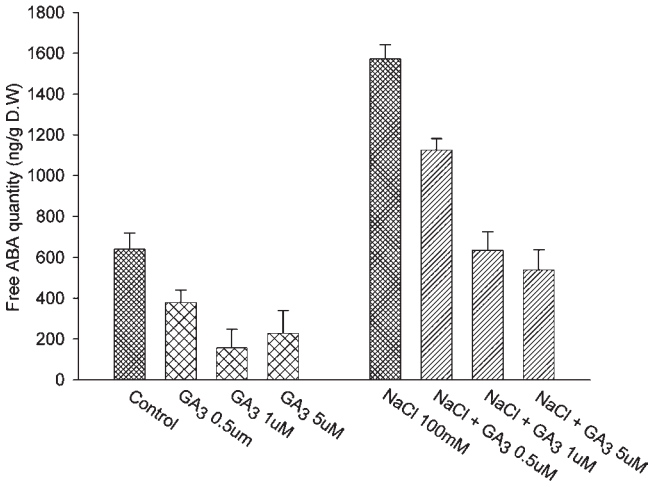

Figure 2. Level of endogenous free ABA content of soybean shoots as influenced by exogenous GA_3 and NaCl.

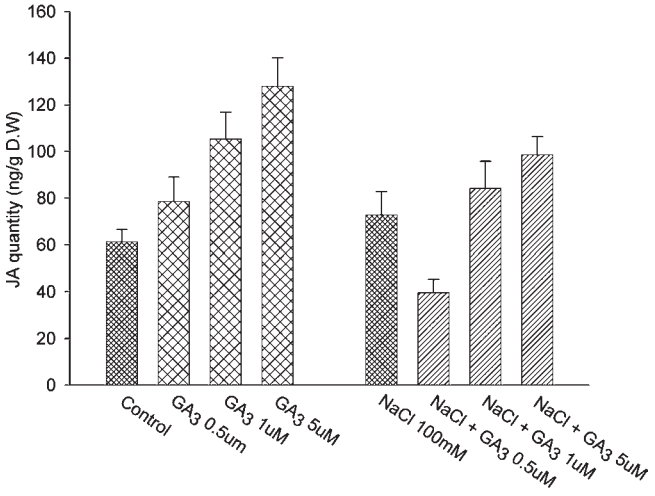

Figure 3. Level of endogenous JA content of soybean shoots as influenced by exogenous GA_3 and NaCl.

GAs increased in such treatments. In salt and GA_3 treated plants, maximum GA_1 and GA_4 contents (3.05 and 13.24 ng/g DW) were recorded in 5.0 μM GA_3 teatments. Current results clearly suggest that GA_3 mitigates the negative effect of NaCl on endogenous gibberellin biosynthesis of soybean.

Influence of Gibberellic Acid on Endogenous Abscisic Acid Contents. ABA analysis showed that endogenous free ABA contents of soybean shoots considerably decreased in GA_3 treatments as compared to those in the control. Minimum ABA content (156.37 ng/g DW) was observed in 1.0 μM GA_3 treated plants. Contrary to GA_3 treatments, the level of ABA was 1572.84 ng/g DW, which was much higher than GA_3 and control (**Figure 2**). Plants that received both NaCl and GA_3 recorded a reduction in free ABA contents, and minimum free ABA content (538.51 ng/g DW) was observed in NaCl treated plants that also received 5.0 μM GA_3 solution.

Influence of Gibberellic Acid on Endogenous JA Contents. JA contents of soybean shoots gradually increased in GA_3 treatments as maximum endogenous JA contents (127.9 ng/g DW) were found in 5.0 μM GA_3 applied plants. In NaCl treated plants, the level of JA was higher than that of the control (**Figure 3**). Plants that received both NaCl and GA_3 exhibit a much lower (39.6 ng/g DW) quantity of JA in 0.5 μM GA_3 treatments.

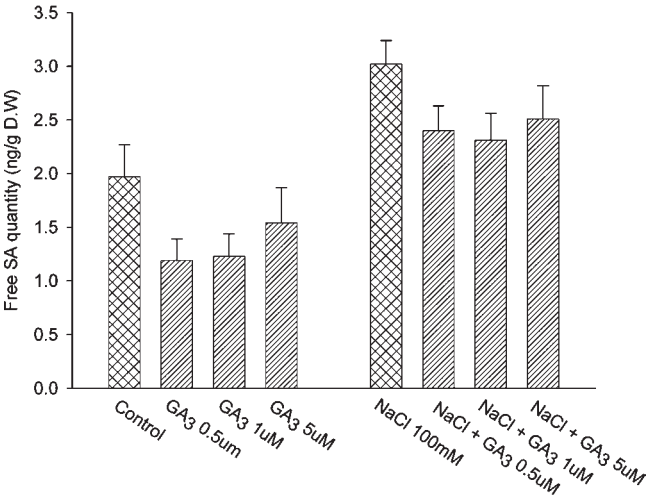

Figure 4. Level of endogenous free SA content of soybean shoots as influenced by exogenous GA_3 and NaCl.

However, the endogenous JA contents markedly increased with elevated GA_3 application.

Influence of Gibberellic Acid on Endogenous SA Contents. The free SA contents of soybean decreased with GA_3 application as compared to the control, although a slight increase was observed with elevated GA_3. The level of endogenous free SA was much higher (3.02 ng/g DW) in NaCl applied treatments as compared to the control (**Figure 4**). An interactive application of NaCl and GA_3 caused a reduction in the free SA level as compared to sole NaCl, but the SA contents were higher than sole GA_3 applied plants. The quantity of free SA in such treatments is not significantly affected by the concentration of GA_3 solution.

DISCUSSION

NaCl induced salt stress adversely affects the growth and development of crops, and the results of our study confirm that all growth variables of soybean drastically decreased with NaCl treatment. Reduction in growth attributes may be due to changes in plant−water relationships under salt stress, which suppress meristem activity as well as cell elongation (*30*). Furthermore, the decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of soybean, which affects growth rate, and is often associated with a decrease in the electron transport activities of photosynthesis (*31*) and inhibition of PSII activity (*32*). Our results pertinent to shoot and root dry biomass did not confirm a previous report (*33*), as we observed that under hydroponic conditions, the root dry biomass parameter was more sensitive to salt stress than shoot dry biomass. A reduction in per unit chlorophyll contents was due to the inhibition of chlorophyll biosynthesis, following an increase in ethylene production brought about by elevated NaCl stress (*34*). The chlorophylase activity also increases during stress conditions (*35*), which suggest that low chlorophyll content could be the result of decreased synthesis and increased degradation under salt stress.

Scientists are working on the improvement of food quality by enhancing the levels of beneficial compounds present in our food. Isoflavones are highly desirable for human health, and soybean cultivars with higher isoflavone contents have been developed over the years, e.g., Aga3 (*36*). Enough literature is available on the favorable role of plant growth regulators (PGR) on isoflavone biosynthesis, although little is known on the effect of these compounds under saline conditions. The current study showed that exogenous GA_3 application markedly improved daizein and genistein contents of soybean and alleviated the negative effect of

salt stress on the biosynthesis of these isoflavones. Previous studies confirmed that GA_3 application increased anthocyanin synthesis and chalcone isomerase enzyme activity in *Petunia hybrida* (*37*). An increase in anthocyanin biosynthesis was correlated with the coordinated appearance of relevant enzymes such as phenylalanine ammonia-lyase (*38*), chalcone flavanone isomerase (*39*), chalcone synthase (*40*), flavanone-3-hydroxylase (*41*), and 3-*O*-flavonoid-glucosyltransferase (*42*). All of these enzymes are involved in the biosynthesis of isoflavonoids (*43*).

Gibberellins have been known as long as plant growth promoters, and the potential of GA_3 to improve crop performance under normal growth conditions is well understood. However, very little has been known on the influence of GA_3 in salt stress environments, as few studies have previously demonstrated the ability of GA_3 to mitigate the adverse effects of salt stress (*44*). We found that GA_3 significantly alleviated the adverse effects of salt stress on growth characteristics of soybean. The chlorophyll content per unit area of soybean was lower in GA_3 treated plants, but as the leaf size was bigger in these plants, the total chlorophyll contents could be higher. In previous reports, GA_3 has been shown to alleviate the effects of salt stress on pigment content, Hill activity (*45*), and water use efficiency (*46*). The current study confirmed earlier reports, which stated that exogenous application of gibberellins alleviates the effect of salinity stress on the growth of *Sorghum* (*47*), wheat (*48*), and rice seedlings (*49*).

Plant growth hormones play a vital role in plant growth, and their responses are of significant importance in understanding the acclimation mechanism of plants. Very little is known about the status of endogenous phytohormones in response to environmental stresses, particularly under salt stress conditions. The current study throws light onto the status of endogenous bioactive GAs, free ABA, JA ,and free SA contents of soybean shoots under various conditions. This study showed that levels of endogenous bioactive GAs markedly increase with GA_3 application but decrease under salt stress. An interactive application seems to rescue GA biosynthesis as the level of bioactive GAs were much higher in those treatments as compared to sole NaCl treated plants. It is known that the predominant GA biosynthetic pathway in most vegetative plant tissues seems to be early-C13-hydroxylation (*50*), though other pathways, especially the non-C13-hydroxylation pathway, are also often present (*51*). Present findings confirm that both early-C13-hydroxylation and non-C13-hydroxylation pathways are operating in soybean and that non-C13-hydroxylation which leads to the formation of GA_4 is the major GA biosynthesis pathway in soybean. Contrary to endogenous bioactive GAs, the endogenous free ABA contents increased under salt stress but declined in sole GA_3 treated plants. ABA is a stress hormone, and its endogenous content usually increases under drought (*52*), salinity (*53*), and cold (*54*) conditions. ABA contents increase under stress, as ABA triggers the acclimation of plants under abiotic stress conditions. A decline in ABA levels clearly suggests that GA_3 application mitigated the stress resulting from NaCl application. Our results confirm a previous report, which stated that NaCl triggers a significant rise in endogenous free ABA contents (*55*).

The current study showed that endogenous JA content gradually increased with elevated GA_3 application but that its level was lower in GA_3 treated salt stressed plants. In the current study, endogenous JA levels may be correlated with plant growth, which was higher in GA_3 treatments. This increase can be explained in the context of vegetative storage protein (VSP) formation, as higher VSP accumulation may have occurred under the influence of elevated GA_3 levels. Previously, it has been reported that endogenous JA content increased with nitrogen nutrition, as N enhanced VSP formation in soybean (*56*), although no information is available on GA_3 and VSP correlation yet. Endogenous JA contents were higher in sole NaCl treated plants as compared to that in the control, which showed that JA is involved in the perception of stress factors. This information confirms a previous report about JA involvement in the perception of stress factors (*57*), while another study (*58*) reported that JA generally increased in response to salt stress. Contrary to endogenous JA, the SA content decreased under the influence of elevated GA_3 while increasing in NaCl treated plants. It suggests that SA biosynthesis was upregulated to strengthen the SAR mechanism under stress conditions. As GA_3 application relieved salt stressed plants, a decline in endogenous SA contents was observed. The role of SA as an antistress hormone is quite evident from SA induced synthesis of heat shock proteins in tobacco plants (*59*), accumulation of wheat lectins (*60*), and fast activation of 48-kD protein kinase in suspension cell culture of tobacco (*61*). However, the mechanism of molecular signaling and its regulation of plant resistance to unfavorable environmental conditions induced by SA are still not clear.

The current study clearly suggests that exogenous GA_3 application reprograms the soybean plant for higher growth, significantly ameliorates the adverse effects of salt stress, and rescues the productivity and quality of soybean. The favorable role of GA_3 in mitigating salinity stress is also evident from the level of isoflavone and endogenous phytohormones of soybean, which provides an important clue for understanding the defense mechanisms of plants against salt stress.

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